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Corrigendum

Corrigendum to "A novel analytical method for in vivo phosphate tracking" [FEBS Lett. 580 (2006) 5885–5893]☆

Hong Gu[a,b], Sylvie Lalonde[a], Sakiko Okumoto[a], Loren L. Looger[a], Anne Marie Scharff-Poulsen[b], Arthur R. Grossman[a], Jens Kossmann[b,c], Iver Jakobsen[b], Wolf B. Frommer[a,*]

[a] *Carnegie Institution, Department of Plant Biology, 260 Panama Street, Stanford, CA 94305, USA*
[b] *Biosystems Department, Risø National Laboratory, Roskilde 4000, Denmark*
[c] *Institute for Plant Biotechnology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa*

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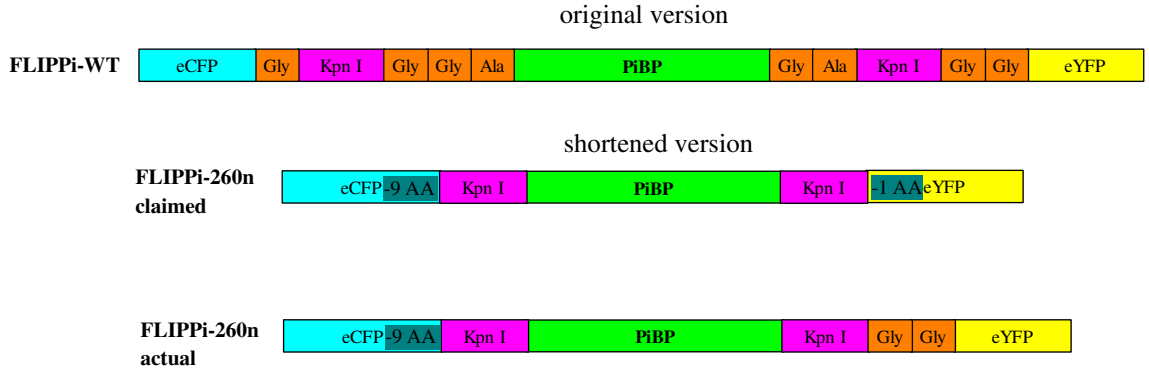
An error occurred in the description of the way the sensors were constructed. Key differences are the length of the linkers, the presence of eYFP instead of Venus and a silent C-to-T transition at Tyr^{313} as compared to the published wild-type sequence [1] (nucleotide sequence position 1633–1635 TAC changed to TAT).

The corrected cloning procedure is described below, the complete corrected sequences and the clones are available at http://www.addgene.org/pgvec1?f=d&cmd=listpl&tvpage=2.

A truncated PiBP (*Synechococcus* strain A; ORF01723), encoding the predicted core of the mature protein without periplasmic leader sequence, was amplified by PCR from genomic DNA of the thermophilic cyanobacterium [1] using the primers 5′-ATT-GGTACCGTAGGATTTCTAACAGCG-3′ and 5′-ATAGGTACCGTTAACGGTGATGGAATC-3′. The PCR fragment was cloned into the *Kpn*I site of FLIPmal-25μ [2] in pRSET-B (Invitrogen, USA), exchanging the sequence encoding the maltose-binding protein with that of PiBP and creating a translational fusion of eCFP-corePiBP-eYFP. The resulting plasmid was named pRSET-FLIPPi-WT corresponding to FLIPPi-840n sensor protein, which showed an affinity of 840 nM for phosphate ("original version"; cf. figure below). A total of 15 amino acids were shortened from the pRSET-FLIPPi-WT sequence: 9 from the C-terminus of the eCFP, 4 and 2 from the left and the right binding protein-fluorophore linker, respectively ("shortened version"; cf. figure below), yielding pRSET-FLIPPi-260n with an affinity for phosphate of 260 nM. Affinity mutants were generated as described in the original text.



References

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*Corresponding author. Fax: +1 6503256857.
E-mail address: wfrommer@stanford.edu (W.B. Frommer).

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